

Antioxidant Activity of Essential Oils Extracted from the Peel of Citrus Limon and That of Tocoblend

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Abstract: *This work concentrated on the valorization of essential oils of Citrus limon. ¶These last were extracted by cold pression (EO1) and by hydrodistillation (EO2), the results gave outputs of 1.02% and 2.18% respectively.¶ The antioxydant activity of extracted oils was studied by the test of DPPH, this powerful radical was effectively reduced by the EO1, the EO2 that by Tocoblend. These results were confirmed by the test of whitening of β -carotene. ¶The two tests have enables to us to classify essential oils extracted among antioxydants powerful compared to Tocoblend.*

Keywords: *Essential oil, Citrus limon, cold pression, hydrodistillation, antioxydant activity, Tocoblend.*

1 Introduction

Lipid oxidation can lead to development of unpleasant rancid or off flavors as well as nutritional value loss. This is one of the main causes of deterioration throughout raw material storage, processing, heat treatment and further storage of final products [1]. Effective antioxidants are added to foods in order to retard or reduce this kind of decline. Synthetic antioxidants have been used for a long time, but their function has recently come into dispute due to a suspected carcinogenic potential and the general rejection of synthetic food additives by consumers. Thus, there is developing appeal to verificate and introduce natural antioxidants as alternatives to the synthetic compounds [2]. Essential oils are known for their natural components, such as monoterpenes, diterpenes, and hydrocarbons with various functional groups. Many other researchers have since reported on antioxidant activities of essential oils in food applications. The purpose of the present study was to evaluate and to compare the in vitro antioxidative properties of essential oil extracts from peels of Citrus limon and the tocoblend.

2 Materials and Methods

2.1 Extraction of Essential Oils

Essential oils were extracted from freshly peels of Citrus limon (Eureka variety). These last were extracted by cold pression (EO1) and by hydrodistillation in a cleveger apparatus for 3h (EO2). The oils were stored in a sealed glass vial in a refrigerator at 4°C until required.

2.2 Antioxidant Activity

DPPH Assay

Bleaching of purple colored methanol solution of DPPH was applied to determine the capability of the related extracts and some pure compounds regarding hydrogen atom or eletron donation. This specrophotometric assay treats constant radical 2, 2-diphenyl-1-picrylhydrazyl (DPPH) as a reagent [3] [4]. In short, different concentrations (50µl) of the extracts, prepared in water and ether, were added to 5ml of a 0.004% methanol solution of DPPH. The absorbance was read against a blank at 517 nm after 30 min incubation at room temperature. Then the following formula was applied to calculate the inhibition free radical DPPH percent:

$$I\% = (A_{\text{blank}} - A_{\text{sample}} / A_{\text{blank}}) \cdot 100 \text{ [5]}$$

A_{blank} is representative for the absorbance of the control reaction (including all reagents except the test compounds) and A_{sample} points to the absorbance of the test compounds.

Extract concentration providing 50% inhibition (IC50) was calculated from the plot of inhibition percentages against extract concentration. All tests were done in triplicate values (mean±SD) of the extracts were compared with those of Tocoblend using student's t-test ($p < 0.05$).

β-Carotene-Linoleic Acid Assay

Antioxidant capacity was determined measuring the volatile organic compounds inhibition and the conjugated diene hydroperoxides arising from linoleic acid oxidation according to the method of Dapkevicius and al [6]. In this respect, a stock solution of β-Carotene-linoleic acid mixture was prepared as follow: 0.5 mg β-Carotene was dissolved in 1ml of chloroform and 25µl linoleic acid and then 200 mg Tween 40 was added. After evaporation of chloroform, 100ml oxygen-saturated distilled water was added with vigours shaking. Then, 2500 µl aliquots were dispensed into the test tubes and the resulted emulsion systems were incubated for 48 h at room temperature. The same procedure was performed for

both Tocoblend (control positif) and blank. In turn, absorbance spectra of the mixtures were compared with those of Tocoblend and blank.

3 Results and Discussion

3.1 Scavenger Effect of Radical DPPH

Table 1 shows the effective concentrations of the substrate that cause 50% loss of activity of DPPH ° and relative antioxidant activity.

Table 1
Effective concentration (EC50) and antiradical power (ARP) of
essential oils of Citrus limon (EO1, EO2) and Tocoblend the DPPH test

Sample	EC50	ARP
Tocoblend	0,0082 ± 0,00036	121,95 ± 0,7993
EO ₁	0,0044 ± 0,0007	227,27 ± 1,7301
EO ₂	0,0054 ± 0,00047	185,185 ± 0,7468

As shown in Table 1, the essential oils of limon are excellent natural antioxidants. They have the capacity to neutralize DPPH by appearing powerful than the Tocoblend. They operate at low doses. Following the EC50 the scanning capability of DPPH ° free radical is classified in the order: EO1 > EO2 > Tocoblend. The antiradical effect of the essential oil could be due to the presence of a great proportion of the phenolic compounds [7]. It is reported that the phenolic compounds can give a hydrogen atom to the free radicals stopping of this fact the chain reaction of propagation during the oxidation lipids process [8]. The phenolic compounds react according to a mechanism proposed by Sherwin [9], the antioxidant yields a hydrogen radical, which can be a transfer of electrons followed, more or less quickly, by a transfer of protons, to give a radical intermediary stabilized from its combined structures mesomeres. According to Kulisic et al. [10], DPPH method is independent of the polarity of the substrate. In the same way, other authors have reported that the polarity of the substrate does not affect the activity of scavenging of the DPPH [11].

3.2 Test of β-Carotene Bleaching

An extract that inhibits or retards the bleaching of β-carotene can be described as a free radical scavenger and as a primary antioxidant [12]. According to several authors, the test for inhibition of the oxidation of linoleic acid coupled to that of β-carotene appears very useful as a mimetic model of lipid peroxidation in biological membranes [13]. The results reported in Table 2 confirms previous results of the neutralization assay of DPPH for the classification of the ability to inhibit oxidation (EO1> EO2> Tocoblend).

Table2
Effective concentration (EC50) and antiradical power (ARP)
of oils extracted (EO1, EO2) and Tocoblend test bleaching β -carotene

Sample	EC 50 (g/l)	ARP
Tocoblend	0,0065 \pm 0,00520	153,84 \pm 0,0908
EO₁	0,0031 \pm 0,0302	322,58 \pm 0,021
EO₂	0,0047 \pm 0,00122	212,76 \pm 0,001

4 Conclusion

The present work aims at exploiting the peel of Citrus limon (Eureka variety) and its recovery in the use of their essential oils through their antioxidant activity and their prices cheaper by appearing in an imported artificial antioxidant (the Tocoblend) at the end of the results, performance evaluation of essential oils of Citrus limon extracted by cold expression (EO1) and hydrodistillation (EO2) are respectively 1.02% and 2.18%. The antioxidant activity determined by DPPH ° test and the test of β -carotene bleaching shows that essential oils extracted are excellent natural antioxidants.

5 References

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